The ever growing complexity of trophoblast epigenetics

Schmidt, Katharina Alexandra

Master's thesis / Diplomski rad

2018

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Zagreb, School of Medicine / Sveučilište u Zagrebu, Medicinski fakultet**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:560219

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Download date / Datum preuzimanja: **2024-03-29**



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UNIVERSITY OF ZAGREB
SCHOOL OF MEDICINE

Katharina Alexandra Schmidt

THE EVER GROWING COMPLEXITY OF TROPHOBLAST EPIGENETICS

GRADUATE THESIS



Zagreb, 2018

This graduate Thesis was made at University of Zagreb, Department of Biology, mentored by Prof. dr. sc. Ljiljana Šerman and was submitted for evaluation 2018.

Mentor: Prof. dr. sc. Ljiljana Šerman

Abbreviations:

DNA	deoxyribonucleic acid
RNA	ribonucleic acid
CpG	5'—Cytosine—phosphate—Guanine—3'
DNMT	DNA methyltransferase
ADP	Adenosine diphosphate
HDAC	Histone deacetylase
ncRNA	non-coding RNA
STB	syncytiotrophoblast
mTOR	mammalian target of rapamycin
mTORC	mammalian target of rapamycin complex
CTB	cytotrophoblast
IGF-2	insulin like growth factor - 2
GLI-2	glioma-associated oncogene - 2
mRNA	messenger RNA
BMI	body mass index
TG	triglyceride
CGI-58	alpha-beta hydrolase 5
FGR	fetal growth restriction
GDM	gestational diabetes mellitus
IUGR	intrauterine growth restriction
BPA	bisphenol - A

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Summary

Title: THE EVER GROWING COMPLEXITY OF TROPHOBLAST EPIGENETICS

Author: Katharina Alexandra Schmidt

Mammalian placentation is closely regulated by the actions of trophoblast cells at the time of implantation. Invasion of trophoblast into uterine tissue is an essential process in placentation and appropriate fetal development. Since human trophoblast is responsible for appropriate fetal growth, it is of major interest in the field of medicine, particularly in understanding how it is influenced by different processes and environmental conditions.

This review is sought to give an insight into the complexity of trophoblast epigenetics and different processes and environmental factors controlling it.

KEYWORDS: Epigenetics, Trophoblast, development, placenta, maternal environment

Sažetak

Naslov: RASTUĆA SLOŽENOST EPIGENETIKE TROFOBLASTA

Autor: Katharina Alexandra Schmidt

Proces implatacije u sisavaca precizno je reguliran djelovanjem stanica trofoblasta tijekom implantacije odnosno njegovom invazijom u sluznicu maternice što je preduvjet razvoja zdrave posteljice i djeteta. Ovakvo invazivno ponašanje trofoblasta, posljedično najviše utjeće na usklađeni rast djeteta, stoga postaje predmet brojih istraživanja na polju medicine s posebnim osvrtom na utjecaj i uvjete okoline. U ovom preglednom radu, nastojali smo dati uvid u složenost epigenetskih procesa u stanicama trofoblasta te različitih faktora koji ga kontroliraju.

Ključne riječi: epigenetika, trofoblast, razvoj, placenta, majčin okoliš

1. Introduction

The field of epigenetics is a rapidly expanding field of contemporary biology, with a significant impact on modern biological processes and therefore of major importance in the field of medicine. [1]. Epigenetics refers to any DNA sequence-independent alteration in the expression of genes and organization of chromatin. [1]. Epigenetics plays a significant role in the functioning of the genome in adults and during development [2], and as such, has attracted significant research interest over the past years. The significant influence of epigenetics has been felt in various fields of contemporary medicine, including placentology, where gene-expression-regulating epigenetic mechanisms have demonstrated a significant influence on gene regulation, as it is evident in the placentation and implantation of trophoblast cells. [3]. The gene regulation associated with epigenetics occurs in various ways, and at different molecular levels. DNA methylation is one of the most frequently researched gene regulations associated with epigenetic mechanisms. [3]. This paper examines the ever-growing complexity of trophoblast epigenetics. Particularly, the paper explores the background of epigenetics, the concepts of DNA methylation, histone modification (acetylation, methylation, ribosylation), and non-coding RNAs. In addition, the paper provides a brief overview about trophoblast development and of the main regulatory pathways.

Epigenetic changes of trophoblast in maternal obesity and diabetes are also examined. Furthermore, effects of environmental stressors are explored, as well as effects of maternal diet on trophoblast development.

2. Epigenetics Background

The term “epigenetic” simply refers to “in addition to changes in genetic sequence.” [4]. Epigenetics has undergone evolution to encompass any process that entails the alteration of gene activity without altering the DNA sequence and can be transmitted to daughter cells. [4]. While epigenetic processes are natural and important to the development and functioning of most organisms, major adverse effects can be seen if they occur improperly.

Epigenetics, just like most of the contemporary scientific discoveries, traces its origin from the 1930s, when Conrad Waddington first used the term *epigenome* to describe the developmental process through which an organism’s phenotype is produced by its genotype. [5]. In 1943, Waddington wrote a seminal paper in which he explained the complex processes involving multiple development-controlling genes. [5]. However, in that seminal paper, Waddington provided no account of the influence of environmental modifiers on gene expression. [6]. The joint work of Jablonka and Lamm [7] attempted to demonstrate a specific new meaning of epigenetics. Jablonka and Lamm are credited for having given epigenetic its first specific definition as the biological mechanisms that give rise to persistent developmental alterations in the activities and effects of genes, without altering DNA sequences from one generation to another. [5].

It was Scott Gilbert’s [9] work, contextualizing Waddington’s ideas of epigenetics, which brought a paradigm shift to the responsibility of genes in influencing the mechanics of development. [5]. Since the enlightening work of this great scientist, the epigenetics landscape has undergone enormous development. Some of the mechanisms associated with the understanding of the processes associated with epigenetic changes include DNA methylation,

histone modification, as well as changes in the chromatin structure as a result of non-coding RNA activity and chromatin re-modelling. [10].

Recently, a strong link between epigenetic regulation and placental development has been established and it is clear that epigenetic regulators, and associated transcription factors, play a critical role in maintaining a healthy pregnancy. [43].

Continued research in the field of epigenetics has been promising, especially in terms of developing novel treatment methods for a wide range of human diseases, most importantly tumorigenesis.

2.1. DNA Methylation

DNA methylation is the best understood epigenetic process and is considered a classical example of epigenetic activity of gene expression during development. The scientific importance of DNA methylation as an epigenetic process and the reason why it is best known, comes mainly from its ease to be studied using nowadays available technologies. [4]. DNA methylation mostly takes place in areas with consecutive occurrence of cytosine bases, where a methyl group (CH3) is added or removed. [4]. The occurrence of DNA methylation was first reported in 1983 associated with human cancer and since then its importance has been recognized in a wide range of human disease. [4].

DNA methylation is a stable epigenetic process that can be inherited from one generation to another through multiple cell divisions and it is very dynamic during the process of mammalian development, maintenance of cellular identity and cell differentiation. [11]. According to Kim and Costello [11,12], the profiles of DNA methylation can play a critical role in classifying lineages as well as controlling the quality of stem cells, such as mesenchymal stem

cells and embryonic stem cells. This is possible because DNA methylation can reflect the original tissue even after a long-term culture. [13,14].

In vertebrates, DNA methylation is confined to the CpG sites. [11]. However, the presence of non-CpG methylation has been reported in pluripotent stem cells. [15,16]. It is estimated that of the twenty-nine million CpG sites that exist in the human genome, between 60% and 80% of them are methylated. [17]. The CpG islands (CGIs), which are characterized by high CpG density, are made up of an estimated 7% of CpG sites. [18]. While CGIs are predominantly resistant to DNA methylation, about 70% of annotated gene promoters are linked to CGIs. [19]. The activity of DNA methylation is accomplished by DNA methyltransferases (DNMTS), which include the main enzymes: DNMT1, DNMT3A, DNMT3B, and DNMT3C [20,21].

Despite the critical role that DNA methylation plays in mammalian development and in disease progression, it remains uncertain whether DNA methylation has a direct function at specific sites. [11]. However, some of the existing technological developments for editing DNA methylation are expected to play an important role in determining site-specific functions of DNA methylation. [25-29]. The role of DNA methylation in identifying the cell of origin is essential in cancer research and in cellular biology. DNA methylation profiles are expected to play a critical role in the medical field, in terms of developing patient type-specific treatment, tumor-type-specific treatment, as well as in regenerative medicine. [11].

2.2. Histone Modification

Histone modifications occur post-transcriptionally, influencing the transcription, as well as repair mechanisms of the DNA.

The genetic potential of DNA is influenced by various epigenetic modifications of histones, like acetylation, methylation, ADP ribosylation, phosphorylation and ubiquitination. [30]. Various epigenetic markers present in the genome demonstrate the significant potential of histone modification. [30]. Epigenetics entails heritable changes in the phenotype which do not cause alterations in the base DNA sequence, and histone modifications are therefore epigenetic regulators of chromatin.

High-order chromatin structure is influenced by the histone code, which determines the contacts between DNA and histones, and between the different histone molecules. [30]. The compartmentalization of the genome into various distinct domains (e.g., transcriptionally active euchromatin and transcriptionally silent heterochromatin) is due to various specific states of histone modification. [31]. The histone code's ability to control the chromatin environment therefore gives it regulatory power over different nuclear processes such as transcription, DNA repair, replication, as well as chromosome condensation. [32].

Besides DNA methylation, histone methylation and histone acetylation are the two other well-explored epigenetic processes. [30]. Epigenetic modifications such as histone modifications, and related nuclear distributions undergo specific alterations during different cellular processes. One of the milestones in cellular biology has been the identification of the various enzymes that initiate epigenetic processes, including histone modification. [33]. The identification of the epigenetic process-initiating enzymes has made it possible to investigate various aspects of epigenetic regulation. [30]. The advancements made in epigenetics research so far has been promising, and the translation of the histone code is expected to lead to more discoveries in related biological processes and disease states. [30]. The knowledge about the influence of epigenetics on genomic reprogramming in the course of embryonic development is expected to

revolutionize the field of regenerative medicine in terms of facilitating the development of novel therapeutic approaches. [34]. In addition, inhibitors of DNMTs and HDACs have demonstrated enormous potential in cancer treatment. [35].

The treatment potential of histone modification has been investigated and reported in various studies. For example, Gavin and Sharma [36] found that modification of the chromatin structure can be a factor in treatment response to schizophrenia. According to their paper [36], histone modification offers an opportunity to develop novel medication for the treatment of schizophrenia.

DNA methylation and histone modification play a critical role in the establishment of patterns of genomic repression during development of mammals. [37]. While certain forms of histone methylation lead to the formation of reversible heterochromatin, DNA methylation on the other hand is associated with stable long-term culture repression. [37]. The modification pathways associated with DNA methylation and histone modification provide an interdependent relationship, which can be mediated by biochemical interactions between DNA methyltransferases and histone methyltransferases. [37]. A study by Cedar and Bergman [37] has found that DNA methylation-histone methylation interrelationships can facilitate the understanding of normal development in mammals as well as tumorigenesis and reprogramming of somatic cell.

2.3. Non-Coding RNAs

Non-coding RNAs (ncRNAs) are all RNA molecules that are not translated into proteins after transcription of the genome. Today we know that more than 60% of the genome are transcribed into ncRNAs and that they play a critical role in the regulation of the human

genome's epigenetic status. [38]. In addition to their involvement in normal physiology, non-coding RNAs are also associated with a wide range of human diseases, including cancer. [38]. According to Morlando and Fatica [38], non-coding RNAs' interaction with epigenetic regulators, their ability to control chromatin topology, as well as their mis regulation, can lead to an aberrant regulation of gene expression, which may act as a contributing factor to tumorigenesis. Since their introduction into the field of epigenetics, thousands of ncRNAs have been identified, and they are for example found to have a direct association with cardiovascular pathologies. [39]. Different classes of ncRNAs with various functions exist. The common ncRNAs and their respective functions are summarised in table 1.

Table 1: Classes and Functions of RNAs. [39]

Classes of ncRNAs	**Examples of ncRNAs**	**Symbols of ncRNAs**	**Description of Functions**
Short-ncRNAs	microRNA	miRNA	Posttranscriptional regulators
	Piwi-interacting RNA	piRNA	DNA methylation and transposon regression
	Short-interfering RNA	siRNA	RNA interference
Long-ncRNAs	Long-intervening ncRNA	lincRNA	Serve as transcription epigenetic regulators.
	Small nucleolar RNA	snoRNA	Facilitation of nucleotide modification.
	Circular RNA	circRNA	miRNA sponging and RNA polymerase II regulators

According to Blignaut [40], the simplified molecular biological dogma, which involves the genetic encoding for protein via mRNA, remains the core of genetics. However, the genomic region that was previously referred to as "junk" DNA has subjected that sequence of events to an extremely complex web of regulation. [40]. Blignaut [40] found that ncRNAs are

not only intricately engulfed by, but also play an active role in influencing the epigenetic processes, such as those that occur during tumorigenesis, in vascular pathologies and in a broad spectrum of other human diseases.

3. Trophoblast Development

Trophoblasts are specialized cells of the placenta and play an important role in implantation and placentation. [41].

Trophoblast development begins a few days after fertilization occurs at the blastocyst stage. The transition from morula to blastocyst is marked by the appearance of a fluid-filled inner cavity and is characterized by cellular differentiation, which gives rise to the embryo (i.e., the inner cells) and the trophoblast (i.e., the surface cells). [41]. The trophoblast is able to proliferate and invade the mother's tissue rapidly, a characteristic shared with malignant cancer cells, but fortunately the processes occurring in the first days of a pregnancy are highly regulated and controlled. After 72 hours upon entering the uterine cavity, the embryo hatches and exposes its outer covering of trophectoderm. [41].

The trophoblast not only plays the physical role of linking the fetus to the mother but also facilitates the alteration of the vasculature in the uterus to allow the provision of adequate blood supply to the fetus. [41].

Implantation of the embryo is highly dependent on differentiation of the trophoblast lineage, which is first evident during the morula stage. [41].

In a way, the development of an embryo can be described as a sequence of controlled epigenetic changes in DNA-methylation patterns over the course of a pregnancy.

Significant influence of epigenetics is observable in a wide range of contemporary medicine, including placentology, in which gene expression-regulating epigenetic mechanisms influence trophoblast implantation as well as placentation. [43]. Epigenetic processes such as DNA methylation play a significant regulatory role in trophoblast implantation and placentation. [43]. In their review, Serman and Dodig [3] demonstrated how alterations in gene methylation can affect trophoblast development and placentation, and thus suggested the potential of trophoblast epigenetics in understating cancer cell invasion and growth. Trophoblast cells not only share their high proliferative rate with malignant cancer cells, but also their loss of contact inhibition, migration and invasion, as well as their ability to control telomerase and tissue vascularization. [66].

The appropriate establishment and organogenesis of the placenta is critical for normal fetal growth and mammalian development. [43]. Proper placental functioning is dependent on syncytiotrophoblast (STB) formation and the invasion of the endometrium by trophoblast cells. [43]. Trophoblast migration and endometrial invasion are akin to tumor metastasis, as they are both characterized by similar molecular mechanisms. [43] However, contrary to the scenario in cancer cells, the initiation of trophoblast migration and endometrial invasion are firmly regulated by a feto-maternal cross-talk, which if not functioning physiologically, can lead to various abnormalities. [43]. The trophoblast is controlled by a range of factors, including hormones and cytokines, and these regulating factors are influenced by transcriptional regulatory networks. [43]

Research has shown that the trophoblast invasiveness is regulated by a complex interplay of various stem cells, as well as signaling pathways. [44]. A number of studies have found haemochorial placentation similarities in humans and rats [44-47]. In this regard, most studies

have used rats as model animals to investigate trophoblast lineage invasiveness. [44]. Consequently, many signaling pathways regulating the differentiation and invasiveness of trophoblast cells have been identified, investigated, and reported about in various studies. Some of the important signaling pathways include: mTOR signaling pathway, IGF-2 signaling pathway, and Hedgehog signaling pathway.

3.1 mTOR Signaling Pathway

Mammalian target of rapamycin (mTOR) has been reported to have a significant regulatory effect on trophoblast differentiation and endometrial invasion. [48].

One example of its importance is the regulation of folate transport in pregnancies, required for methylation reactions, DNA repair and *de novo* DNA synthesis. [48].

Even though we know that the fetus depends entirely on placental transfer of folate and that the lack of it has devastating effects on the development of an embryo, like structural malformations and intrauterine growth restriction, the molecular mechanisms that are responsible for the placental folate transportation remain unknown.

Using human trophoblast cells, the hypothesis that mTORC1 and mTORC2 are regulators of placental folate transportation, has been tested. [48]. The results show that both, mTORC1 and mTORC2 are significant positive regulators of the placental folate transportation. According to [48], mTOR's signaling regulation of the placental folate transportation provides a direct link between the functions of placenta, fetal programming, and gene methylation. As mentioned above, fetal development and growth depends on the mother's folate availability and by regulating its transport, mTORC1 and 2 signaling could be a very important connection between

maternal nutrition and fetal growth. [48]. mTOR is believed to serve as a nutrient sensor, having the ability to be regulated by growth factor signaling in multicellular organisms. [49]. Another important function of mTOR was researched by Wen [49] who investigated trophoblast cell proliferation in reaction to angiopoietin-2 (Ang-2), which is specifically expressed in the placenta. Here, the mTOR signaling pathway was used to induce trophoblast proliferation. The ultimate finding of this study shows the importance of mTOR in regulating trophoblast cell proliferation as well as endometrial invasion and therefore, it is essential in determining the success of every pregnancy. [49].

3.2. IGF-2 Signaling Pathway

Insulin-like growth factors 2 (IGF-2) enhances human first-trimester cytotrophoblast (CTB) proliferation and survival by signaling via the insulin-like growth factor 1 receptor (IGF1R). [50]. Phenotypic characterization of IGF-2 null mice established the potential of IGF-2 in regulating placentation, as well as fetal and postnatal mammalian growth. [51]. For instance, Harris [50] investigated trophoblast proliferation and survival in response to IGF-2 signaling. The findings indicated that IGF-2 had a regulatory effect on trophoblast proliferation and endometrial invasion. In another study that came to a similar conclusion, Chen [52] investigated the effect of IGF-2 on the proliferation of human trophoblast *in vitro*. Ultimately, the results established that IGF-2 enhances extra villous cytotrophoblasts (EVCTs) proliferation and also inhibits apoptosis. [52]. The study concluded that the IGF-2 is an important signaling pathway for regulating the proliferation of trophoblasts during the early stages of human pregnancy *in vitro*, therefore indicating the significance of the IGF-2 signaling pathway in regulating trophoblast proliferation and endometrial invasion in every pregnancy. [52].

3.3. Hedgehog Signaling Pathway

The hedgehog proteins, namely Sonic hedgehog, Indian hedgehog and Desert hedgehog, have specific roles in various processes of organogenesis in humans. [53].

Cell-to-cell fusion in the placenta to form STBs - *syncytialization* - plays a critical role in ensuring normal development and efficient functioning of the human placenta. [53]. Hedgehog signaling proteins are largely expressed in the human placental villi. [53]. In this regard, Tang et al. investigated the potential of Hedgehog signaling pathway in regulating trophoblast fusion and its need for synthesis of human placental hormones. The study established that Hedgehog/GLI2 (glioma-associated oncogene) had a significant signaling-influence in inducing syncytialization and trophoblast fusion. Based on these results, it can be concluded that Hedgehog signaling, through GLI2, plays a critical role in enhancing mammalian placental development, as well as in pregnancy maintenance. [53].

In another study, Lu [54] hypothesized that Estrogen-related receptor β could enhance the modulation of the Hedgehog signaling pathway and consequently affect Hedgehog-driven gene expression. The findings of that study indicated that Hedgehog signaling has a significant impact on several Hedgehog-responsive mRNAs, including *Hoxd8*, and *Hsd11b1*. [54]. The results indicate the significance of the Hedgehog signaling pathway in regulating trophoblast proliferation and endometrial invasion.

Hedgehog proteins play a vital role in cell growth, cell survival and cell fate decision, being one of the most important intracellular signals, especially regarding implantation and successful placental maintenance. [53].

IGF-2, mTOR, and Hedgehog serve as important signaling pathways, all essential for healthy development of an embryo, mainly by regulating trophoblast proliferation and invasion of the endometrium.

4. Epigenetic Changes of Trophoblast in Maternal Obesity

Obesity is a major health problem in the developed world, affecting more and more adults and an increasing number of children. Women of reproductive age are increasingly overweight (BMI = 25-30 kg/m2) or obese (BMI > 30kg/m2); consequently, the number of obese pregnant women is increasing as well. [55]. Both maternal obesity and excessive gestational weight gain cause an adverse intrauterine environment and are a major risk contributor for developing metabolic diseases, but also program the fetus in ways that may become apparent later in life. [55]. An obese maternal environment is characterized by hyperlipidemia and chronic state of inflammation, as well as increased oxidative stress, compared to a normal pregnancy. [69].

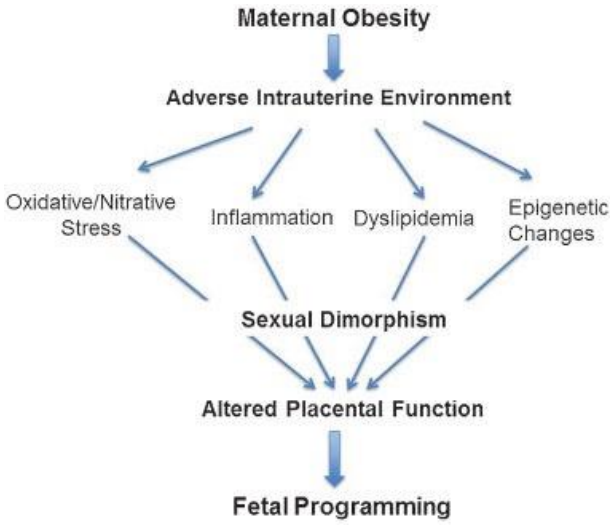


Figure 1: Mechanisms linking maternal obesity to placental dysfunction and developmental programming. [64].

Pregnancy is a state of profound metabolic changes; maternal obesity strongly contributes to an adverse intrauterine environment and is often linked to an impaired metabolic status of the offspring and the mother. [56]. The effects of an adverse maternal environment are transmitted to the fetus via the placenta. According to Hirschmugl, the adverse effects of impaired metabolic functions are often attributed to epigenetic changes in *utero*. However, the associations between these adverse effects and in utero epigenetic changes are insufficiently understood. Due to the uncertainties and lack of clear-cut evidence on the linkage between maternal obesity and the impaired metabolic status of the offspring and mother, Hirschmugl [56] studied 73 lean and obese mothers with healthy pregnancies. The study measured certain metabolic parameters (e.g., gene expression, gene protein quantification) from the recruited women's maternal venous blood samples, which were then correlated with maternal parameters and immunolocalized in placental segments. [56]. The results showed that triglyceride (TG) content in the placental tissues of lean participants were much lower than in obese participants. [56]. In addition, among examined genes, six exhibited a significant positive correlation ($P < 0.05$) with maternal pre-pregnancy BMI. Protein content was also found to be higher ($p < 0.001$) in placentas of obese participants compared to lean participants and a positive correlation was established between CGI-58 protein levels and maternal insulin levels, adversely causing hypoglycemia in the fetus in utero and contributing to development of insulin resistance later in life. [56].

Accordingly, [56] suggested that the up-regulation of CGI-58, which was identified as a master regulator of TG hydrolysis, causes the turnover of intracellular lipids in the placenta of obese women. CGI-58 is tightly regulated by the mother's metabolic factors and, therefore, it can

be concluded that maternal obesity is responsible for the modulation of the expression of lipid-related genes within the human placenta. [56].

Panchenko [57] highlighted that the epigenetic machinery and gene expression, particularly the histone acetylation pathway, is highly sensitive to maternal obesity. The transcriptional changes induced by maternal obesity can cause alterations in the placenta as well as in the hepatic epigenome, and cause fetal growth restriction (FGR), particularly in obese women. [57]. According to Panchenko, pre-conceptional weight loss is necessary for ensuring proper fetal growth, but certain effects of pre-pregnancy obesity can still be retained in the offspring's phenotype. [57].

The existence of chronic low-grade inflammation in obese women leads to an inflammatory in utero environment and therefore adverse outcomes for the physiologic development of the embryo. [64].

5. Epigenetic Changes of Trophoblast in Maternal Diabetes

Diabetes is not only an increasingly present problem in nowadays society, the number of pregnancies accompanied by gestational diabetes mellitus (GDM) is increasing as well. In developed countries approximately every 10^{th} pregnancy is complicated by GDM and many of them remain unrecognized and therefore untreated, partly due to a lack of universal screening programs. [65].

Due to an increased need for food supply to the fetus in the late trimester, the mother can develop a moderate peripheral insulin resistance, creating a diabetogenic situation. The resulting intrauterine hyperglycemia not only leads to an increase in perinatal morbidity/mortality but also poses the risk of exposing the offspring to the development of chronic diseases later in life. [58].

Over- and under-nutrition can lead to persistent epigenetic changes in the offspring's genome. These changes consequently influence metabolism, neuroendocrine functions and energy homeostasis. [58]. According to [58], the success of a pregnancy is determined not only determined primarily by the outcome at birth, but also by the status of postnatal health.

Haertle et al. showed that intrauterine exposure to GDM leads to epigenetic modifications and poses a chronic risk of complex disorders to the offspring. [59]. They conducted a genome-wide comparison of methylation patterns of fetal cord blood (FCBs) taken from mothers with GDM with those from mothers without GDM. The study found that the effects of GDM on FCB methylation were predominant in women who presented with insulin-dependent GDM, and who had been diagnosed with more severe metabolic phenotypes. [59]. However, and according to the findings of the study, FCB methylation of women who presented with dietetically treatable GDM was less affected. [59].

The findings show a clear correlation between maternal diabetes, particularly GDM, and epigenetic changes in the trophoblast. Despite the observable small effect size associated with the FCB methylation, the effect of FCB methylation on genes is significant and detrimental to the exposed offspring. [59]. According to Haertle et al. the identified genes act as primary vehicles for transmitting the effects of GDM to the succeeding generation. Even though the mechanisms responsible for long-term morbidity risk in the offspring are still poorly researched and understood, epigenetics is thought to be a key player in the process. [59]
In addition, the genes may act as sources of essential biomarkers for diagnosis, prognosis and treatment of harmful prenatal conditions. [59]

6. Effects of Environmental Stressors on Trophoblast Epigenome

The identification of the impact of environmental stressors on human health lies at the core of life sciences and biomedical research. [60]. The recognition that exposure to adverse environmental conditions could lead to DNA mutations forms the idea of environmental risk assessment and prevention. [60]. The human placenta and its various functions play a critical role in ensuring healthy and desirable reproductive outcome. [61]. According to Robins [61], the environment within a pregnant woman lives in or is exposed to can impact and influence the placental functions. Some environmental stressors can lead to the alteration of the appropriate genetic programming necessary for a sustainable pregnancy and normal, healthy fetal development. [61].

The alteration of genetic programming can be caused by environment-exposure-related epigenetic alterations. [61]. Research continues to link epigenetic alterations not only to successful pregnancies, but also to other significant reproductive outcomes. [61]. Some of the reproductive outcomes that can result from environmental exposures include early stage pregnancy loss, congenital syndromes (such as Beckwith-Wiedemann syndrome), IUGR, as well as preeclampsia and pre-term birth. [61].

Trophoblast cells are responsible for connecting the embryo to the human uterus and serve as interface between the fetus and the mother. The hormonal mechanisms associated with trophoblast activity are influenced by physiologic alterations associated with pregnancy, most of which are dependent on the status of the pregnancy. [61]. Therefore, to ensure a successful maintenance of the pregnancy it is fundamental to ensure normal trophoblast development. Factors that can interfere with normal trophoblast development are for example endocrine disruptors, such as xenoestrogens and antiandrogenes, and even altered intrauterine oxygen

levels can play a critical role. [61]. This can be further explained on the example of BPA. BPA is one of the xenoestrogens and the exposure risk today is widely connected to use of plastic bottles, where especially when heated up, the chemicals leach into the content of the bottles. BPA's toxicity and endocrine disruption is mainly due to its binding to estrogen receptors, thus interfering with endogenous estrogen, and leading to disruptions of the reproductive system, brain and behavior. [68].

In a survey from 2004, BPA could be detected in more than 95% of Americans over the age of six. [61].

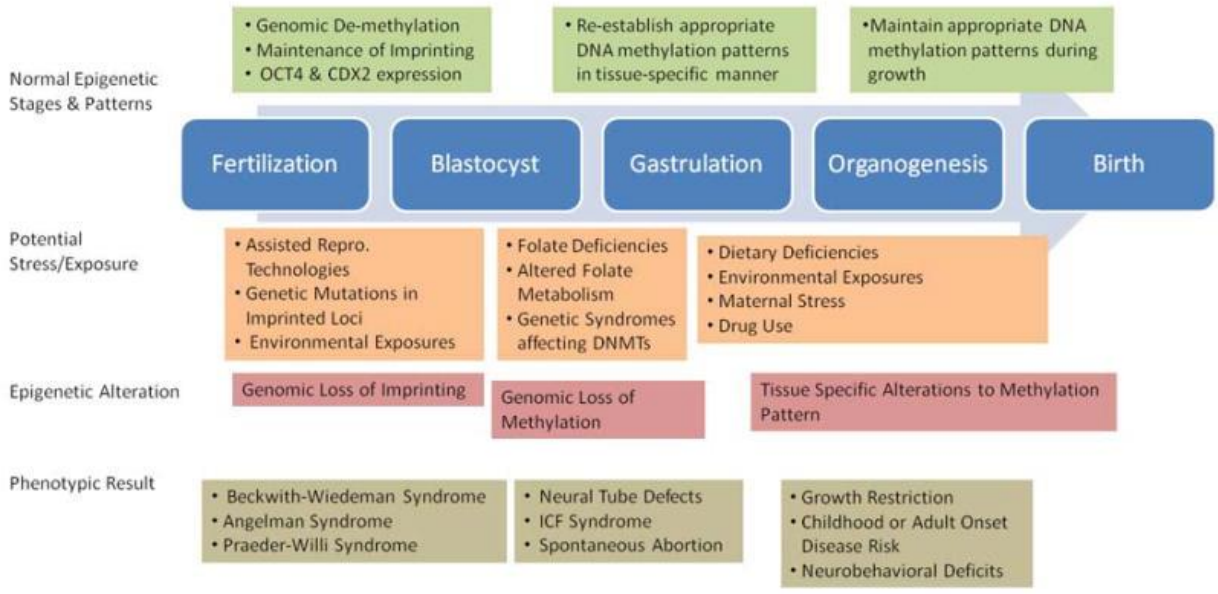


Figure 2: Model of appropriate epigenetic programming throughout gestation and the potential epigenetic effects of environmental factors and their downstream consequences. [61].

This implies that monitoring and preventing environmental hazards, like BPA, to which a pregnant woman is exposed, are the key components in maintaining a healthy and successful pregnancy.

7. Effects of Maternal Diet on Trophoblast Development

Maternal nutrition plays a major role in fetal and placental development, partly by providing the necessary nutrients for successful growth of the fetus's genomic structure and function and also by its impact on the placenta and its ability to support the fetus with required conditions for a successful development. The behaviour of trophoblast cells during the time of implantation has a significant influence on mammalian placentation. [62]. Appropriate fetal growth and development is greatly influenced by the maternal nutrition the offspring is exposed to. [62]. In recent years, regulation of fetal growth and development has been increasingly interesting for epidemiological and human observational models, to show a link between adversely changed fetal growth and predispositions to cardiovascular and metabolic disease later in life. [62]. Thanks to the significant similarities between rodent and human preimplantation embryo development, implantation and placental morphology, it hast been possible to use mice and rats as biologically applicable models in researching the effects of maternal diet on developing placenta, as well as on fetal and child development and health. [62].

A study conducted by Watkins using a mouse maternal diet, as representative of a human maternal diet, found that a mouse maternal diet that is low in calories decreased the growth of the offspring and leads to genome-wide hypomethylation, whereas a diet high in fat induced placental gene expression changes that regulate epigenetic modifications and predisposed the offspring to adult metabolic and cardiovascular conditions. Decreasing a diets protein contents in rodents lead to apoptosis, growth inhibition and epigenetic modification, especially effecting mTORC1. In contrast, if fed with high protein diet exclusively during preimplantation, mice displayed enhanced postnatal growth and adiposity, as well as hypertension and vascular dysfunctions in adult offspring. [62]. According to Watkins et al. enhanced early postnatal

growth was found out to be a significant predictor of adiposity, cardiovascular disease and behavioral changes in adults. They also found that trophoblast was capable of modifying its growth and phenotype for purpose of regulating and ensuring proper growth of the fetus in cases of poor maternal nutrition. However, as mentioned above, these adaptive mechanisms will become maladaptive if the changes persist for too long and eventually predispose offspring to adult-onset disease. [62].

Therefore, unfavorable maternal diet can expose the fetus to a wide range of diseases, including hypertension, obesity, and metabolic syndrome. [63]. Kappen et al. demonstrated that pregnancy-specific diets are detrimental to the health and well-being of the offspring.

Conclusion

This review seeks to develop an understanding of the ever-growing complexity of trophoblast epigenetics. Continued research in the field of epigenetics has been promising, in terms of understanding a wide range of human disease and more importantly developing novel treatment methods. The significant influence of epigenetics is evident in human placentology, where it has been found that gene expression-regulating epigenetic mechanisms influence trophoblast implantation and placentation and, therefore, determine the success of a pregnancy. Three main regulators of epigenetic mechanisms - non-coding RNAs, DNA methylation, and histone modification - have proven to be of importance in understanding the beginning of life, as well as regenerative medicine and various other domains of science.

The trophoblast not only plays the physical role of linking the fetus to the mother but also facilitates the alteration of vasculature in the uterus to allow the provision of adequate blood supply to the fetus.

The results reviewed here indicate the significance of environmental factors contributing to fetal developmental outcome and, subsequently, to the health of an adult.

It is not yet fully understood how many different mechanisms exist that influence epigenetics and, thus, future research should focus on exploring epigenetic regulations, especially in the field of placentology, as well as cancer treatments.

Acknowledgement

I would first like to thank my mentor Prof. dr. sc. Ljiljana Šerman from the Department of Biology at our Medical University. She provided me with key information and was always ready to help me and answer my questions to comprehend such a complex topic.

Furthermore, I want to thank the University of Zagreb, to give me the opportunity to follow my dream and finish the medical studies, surrounded by amazing people from different parts of the world, in an incredible country. I will always keep this experience close to my heart.

My final gratitude goes to my family and my life partner for their steady support and their love in all aspects of my life.

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Biography

I was born in the south of Germany in 1991. As a daughter of a Biology professor and a GP, I knew quite soon that I would like to pursue a career in science.

Always preferring scientific subjects in school, I finally decided to study medicine. Together with a friend, I applied for medicine in Hungary. This is where I spent the first two years of medical studies.

In Hungary, more precisely Pecs, I met my boyfriend with whom I decided to continue medical studies together in Zagreb, in their English program.

Now we will move back to Germany, excited to start our specialization and curious for the experiences we will encounter in the upcoming years.